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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/520,538	03/08/2000	Arlene A. Wise	S-91,714	2050

7590   08/13/2002

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EXAMINER
STEADMAN, DAVID J

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 08/13/2002   *15*

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 07-01)

-- *The MAILING DATE of this communication appears on the cover sheet with the correspondence address* --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE **3** MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☐ Responsive to communication(s) filed on _____ .

2a) ☐ This action is **FINAL**.   2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) *1 and 8* is/are pending in the application.

   4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) *1 and 8* is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

   Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) ☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

   If approved, corrected drawings are required in reply to this Office action.

12) ☒ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   a)☐ All b)☐ Some * c)☐ None of:

   1. ☐ Certified copies of the priority documents have been received.

   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .

   3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

   * See the attached detailed Office action for a list of the certified copies not received.

14) ☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

   a) ☐ The translation of the foreign language provisional application has been received.

15) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .

4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .

5) ☐ Notice of Informal Patent Application (PTO-152)

6) ☐ Other: _____ .

DETAILED ACTION

Status of the Application

The request filed on 05/07/02 for a Continued Prosecution Application (CPA) under 37 CFR 1.53(d) based on parent Application No. 09/520,538 is acceptable and a CPA has been established. An action on the CPA follows.

Claims 1 and 8 are pending in the application.

Receipt of a computer-readable form of the sequence listing, a paper copy thereof, and a statement that the paper copy of the sequence listing and computer-readable form are the same in Paper No. 12, filed 05/23/02, is acknowledged.

Receipt of formal drawings in Paper No. 13, filed 05/23/02, is acknowledged.

The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Oath/Declaration

1.   The oath or declaration remains defective. See Paper No. 4 for details. It is noted that applicants agree to submit a corrected declaration upon allowance of claimed subject matter.

Drawings

2.   The drawings have been reviewed and approved by the Draftsperson.

Claim Rejections - 35 USC § 112, Second Paragraph

3.   Claims 1 and 8 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

4.   Claims 1 and 8 are indefinite in the recitation of "response" in lines 1, 3, and 14 as it is unclear from the claims and the specification as to the meaning of the term "response". One of skill in the art would recognize that bacteria can respond to phenols in a variety of ways. For example, a bacterial

response to the presence of phenols may be increased or decreased growth, increased or decreased gene expression, a change in colony size or shape, etc. It would appear from the claims and the specification that the intended "response" is activated expression of genes encoding metabolic enzymes. Therefore, an enhanced "response" has been interpreted by the examiner as an enhanced expression of genes encoding metabolic enzymes and the claims have been examined accordingly. It is suggested that applicants clearly identify the intended response.

5.   Claims 1 and 8 recite the limitation "the bacterial DNA encoding the regulatory protein" in line 11. There is insufficient antecedent basis for this limitation in the claim.

6.   Claims 1 and 8 are indefinite in the recitation of "bacterial DNA". It is unclear from the claim as to whether the "bacterial DNA" is genomic DNA or whether a bacteria comprises the "bacterial DNA" on a vector. One of skill in the art would recognize that E. coli do not carry an endogenous gene encoding DmpR, MopR, PhhR, PhlR, XylR, or TbuT regulatory proteins. Therefore, it would appear that the term "bacterial DNA" is meant to refer to a DNA carried on a vector. However, as written, the term remains unclear. It is suggested that applicants clarify the meaning of the term.

Claim Rejections - 35 USC § 112, First Paragraph

7.   Claims 1 and 8 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of enhancing reporter gene expression in P. putida, Acinetobacter, and E. coli bacteria in response to phenols or substituted phenols by mutating the sensor domain of the respective recited regulatory protein that activates reporter gene expression by mutagenic PCR or gene shuffling, does not reasonably provide enablement for a method of enhancing *any* response of P. putida, Acinetobacter, and E. coli bacteria to phenols or substituted phenols by mutating the sensor domain that activates expression of *all* genes encoding *any* metabolic enzyme by mutagenic PCR or gene shuffling. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are

summarized in *In re* Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the

quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the

presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art,

(6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the

breadth of the claim(s).

Claims 1 and 8 are so broad as to encompass a method of enhancing *any* response of P. putida,

Acinetobacter, and E. coli bacteria to phenols or substituted phenols by mutating the sensor domain that

activates expression of *all* genes encoding *any* metabolic enzyme by mutagenic PCR or gene shuffling.

The scope of the claims is not commensurate with the enablement provided by the disclosure with regard

to the extremely large number of bacterial responses and genes encoding metabolic enzymes whose

expression can be activated by a sensor domain of the recited regulatory proteins. In this case the

disclosure is limited to a method of enhancing *reporter gene expression* in P. putida, Acinetobacter, and

E. coli bacteria in response to phenols or substituted phenols by mutating the sensor domain that

activates reporter gene expression by mutagenic PCR or gene shuffling.

The specification does not support the broad scope of the claims which encompass a method of

enhancing *any* response by mutating the sensor domain that activates expression of *all* genes encoding

any metabolic enzyme by mutagenic PCR or gene shuffling because the specification does not establish a

rational and predictable method for enhancing *any* bacterial response by mutating the sensor domain

and/or a rational and predictable scheme for activating expression of *all* genes encoding *any* metabolic

enzyme by mutating the sensor domain. Neither the specification nor the prior art provides guidance or

working examples for practicing a method of enhancing *any* response by mutating the sensor domain

that activates expression of *all* genes encoding *any* metabolic enzyme by mutagenic PCR or gene

shuffling. One of skill in the art would recognize that mutations to the sensor domain resulting in the

enhancement of any bacterial response or activate expression of all genes encoding any metabolic

enzyme are highly unpredictable.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any number of amino acid modifications of any . The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re* Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re* Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 103

8.   Claim 1 is rejected under 35 U.S.C. 103(a) as being unpatentable over Pavel et al. (J Bacteriol 176:7550-7557) in view of Willardson et al. (Appl Environ Microbiol 64:1006-1012), Minshull et al. (US Patent 5,837,458) and Cadwell et al. ("Mutagenic PCR" pp 583-589 in "PCR Primer, A Laboratory Manual", Cold Spring Harbor Laboratory Press, 1995). Claim 1 is drawn to a method of enhancing a response of *Pseudomonas putida, Acinetobacter,* and *Escherichia coli* bacteria to phenols and substituted phenols said bacteria having a regulatory protein selected from DmpR, MopR, PhhR, PhlR, XylR, and TbuT with a sensor domain for detecting phenols, a DNA binding region, and a transcriptional activation region, the method comprising the steps of removing the DNA encoding the sensor domain, subjecting the sensor domain DNA to mutagenic PCR or gene shuffling, ligating the mutant sensor domain into the DNA encoding the regulatory protein, and testing the bacteria for enhanced response to said phenols.

Pavel et al. teach that the sensor domain (referred to as the "A domain" by Pavel et al.) of DmpR binds (methyl)phenols resulting in transcriptional activation (page 7550, abstract and page 7556, paragraph 2) and that DmpR responds to (methyl)phenols with the magnitude of transcriptional response differing depending on the position of the methyl substituent (page 7550, abstract) and further teach that the response to *para*-substituted phenolic compounds (i.e., 4-methylphenol and 3,4-methylphenol) is relatively poor (page 7550, Introduction). Pavel et al. further teach a method of mutating DmpR by chemical mutagenesis (pages 7551-7552 under *Construction of P_o Km selection strain and isolation of*

DmpR specificity mutant) to generate a mutant DmpR that, when expressed in *P. putida* with a

chromosomally inserted reporter gene (page 7552, under *Construction of P₀ luxAB reporter strain and*

luciferase assays), exhibits increased luciferase expression relative to wild-type DmpR in response to 4-

methylphenol, 3,4-dimethylphenol, and 4-ethylphenol (page 7554, Fig 3) and that sequencing the gene

encoding the DmpR mutant revealed a mutation at codon 135 (page 7554, under *Genetic selection of an*

effector specificity mutant, DmpR-E135K) of the sensor domain of DmpR (amino acids 1-211; page 7556,

paragraph 2). Pavel et al. also teach that a comparison of the responses of the wild-type and mutant

DmpR to various phenolic derivatives suggests that, in addition to the increased responses of the mutant

to 4-methylphenol, 3,4-dimethylphenol, and 4-ethylphenol, the mutant DmpR mediates responses to

phenol, 2-methylphenol, and 3-methylphenol to similar extents as wild-type DmpR (page 7554, under

Effector profile comparison of DmpR⁺ and DmpR-E135K), suggesting that the mutant DmpR response to

4-methylphenol, 3,4-dimethylphenol, and 4-ethylphenol is enhanced relative to wild-type DmpR without

altering the function of the other domains, i.e., DNA binding and transactivation domains. Pavel et al.

further teach that DmpR shares significant sequence similarity with XylR, a *Pseudomonas* regulator of

toluene and xylene catabolism (page 7550, Introduction, paragraph 1) and that mutations at residues

135 and 172 of DmpR and XylR, respectively, result in the ability of the proteins to recognize a novel

effector compound (page 7556, left column, bottom).

Pavel et al. teach the limitations of claim 1 except for mutating *only* the sensor domain of DmpR

by PCR mutagenesis.

Willardson et al. teach a biosensor using *Escherichia coli* expressing XylR that responds to

toluene and derivatives thereof by luminescence proportional to the concentration of toluene or

derivatives thereof present in a medium. Willardson et al. further teach "the development of this

biosensor for toluene and its derivative compounds demonstrates the feasibility of constructing similar

biosensors with specificity for other organic contaminants by using their corresponding transcriptional

activators" (page 1011, bottom – 1012, top). Willardson et al. also suggests using other bacterial strains

as biosensors (page 1012, top).

Minshull et al. teach mutagenesis can be used (also referred to as recursive sequence recombination) to alter the specificity of biosensors for detection of different chemicals, i.e., analogues of the normal effector compound (column 35, middle). Mishull et al. teach a method for mutating a biosensor for detecting a compound of interest (columns 3 and 4).

Cadwell et al. teach a mutagenic PCR method of randomly mutating a nucleic acid in order to generate a library of mutant nucleic acids (page 584 under *Protocol*). Cadwell et al. further teach that using these mutants, one can apply a screening method to isolate individual clones that exhibit a particular property. (page 583, Introduction, paragraph 2).

Also, at the time of the invention, it was known in the art to mutate a specific domain of a protein without mutating other domains. Minshull et al. provide an example of mutating only a specific catalytic domain of a polyketide synthase, which contains several different catalytic domains, to optimize only a desired catalytic activity. As such, it would have been obvious to one of ordinary skill in the art to mutate *only* the sensor domain as described by Pavel et al., because one of ordinary skill would have recognized that, in order to broaden the binding specificity of the sensor domain to recognize other phenolic compounds, one need mutate *only* the sensor domain, and not the DNA binding or transactivation domains. Furthermore, as described above, Pavel et al. disclose that mutation to *only* the sensor domain provides altered effector specificity for 4-methylphenol, 3,4-dimethylphenol, and 4-ethylphenol without disrupting the response to the original effector compounds, i.e., phenol, 2-methylphenol, and 3-methylphenol.

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Pavel et al. with Willardson et al., Minshull et al., and Cadwell et al. for a method of enhancing the response of *P. putida* or *E. coli* to phenols and substituted phenols by removing the DNA encoding the sensor domain of DmpR or XylR, mutating the respective sensor domain by mutagenic PCR, ligating the mutant sensor domain into the DNA encoding the respective regulatory protein, and testing the bacteria for enhanced response to said phenols. One would have been motivated to mutate *only* the sensor domain of the regulator proteins of claim 1 because of the teachings of Pavel et al. who taught that the

binding specificity of regulatory proteins can be broadened by mutating only the sensor domain and not the DNA binding and transcriptional activation domains. One would have been motivated to use mutagenic PCR to mutate the sensor domain because of the teachings of Cadwell et al. who taught that by using mutagenic PCR, one can create a library of mutants to isolate those with desirable properties. One would have a reasonable expectation of success for a method of enhancing the response of *P. putida* or *E. coli* to phenols and substituted phenols by removing the DNA encoding the sensor domain of DmpR or XylR, mutating the respective sensor domain by mutagenic PCR, ligating the mutant sensor domain into the DNA encoding the regulatory protein, and testing the bacteria for enhanced response to said phenols because of the results of Pavel et al., Willardson et al., Minshull et al., and Cadwell et al. Therefore, claim 1, drawn to a method of enhancing the response of the recited bacteria to phenols and substituted phenols by removing the DNA encoding the sensor domain of the recited regulator protein, mutating the sensor domain by mutagenic PCR and ligating the mutant sensor domain into the DNA encoding the regulatory protein, and testing the recited bacteria for enhanced response to said phenols would have been obvious to one of ordinary skill in the art.

9.   Claim 8 is rejected under 35 U.S.C. 103(a) as being unpatentable over Pavel et al. (J Bacteriol 176:7550-7557) in view of Willardson et al. (Appl Environ Microbiol 64:1006-1012), Minshull et al. (US Patent 5,837,458) and Stemmer (Nature 370:389-391). Claim 8 is drawn to a method of enhancing a response of *Pseudomonas putida, Acinetobacter,* and *Escherichia coli* bacteria to phenols and substituted phenols said bacteria having a regulatory protein selected from DmpR, MopR, PhhR, PhlR, XylR, and TbuT with a sensor domain for detecting phenols, a DNA binding region, and a transcriptional activation region, the method comprising the steps of removing the DNA encoding the sensor domain, subjecting the sensor domain DNA to gene shuffling, ligating the mutant sensor domain into the DNA encoding the regulatory protein, and testing the bacteria for enhanced response to said phenols.

Pavel et al., Willardson et al., and Minshull et al. disclose the teachings as described above. Pavel et al. teach the limitations of claim 8 except for mutating *only* the sensor domain of DmpR by gene shuffling.

Stemmer teaches a method of *in vitro* homologous recombination of pools of selected mutant genes by random fragmentation and PCR reassembly, i.e., gene shuffling (page 390, Fig 1) and teach that one would use gene shuffling over mutagenic PCR because mutagenic PCR is not combinatorial and thus, is more limited in the number of possible mutations (page 389, abstract and page 390, right column).

Also, at the time of the invention, it was known in the art to mutate a specific domain of a protein without mutating other domains. Minshull et al. provide an example of mutating only a specific catalytic domain of a polyketide synthase, which contains several different catalytic domains, to optimize only a desired catalytic activity. As such, it would have been obvious to one of ordinary skill in the art to mutate *only* the sensor domain as described by Pavel et al., because one of ordinary skill would have recognized that, in order to broaden the binding specificity of the sensor domain to recognize other phenolic compounds, one need mutate *only* the sensor domain, and not the DNA binding or transactivation domains. Furthermore, as described above, Pavel et al. disclose that mutation to *only* the sensor domain provides altered effector specificity for 4-methylphenol, 3,4-dimethylphenol, and 4-ethylphenol without disrupting the response to the original effector compounds, i.e., phenol, 2-methylphenol, and 3-methylphenol.

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Pavel et al. with Willardson et al., Minshull et al., and Cadwell et al. for a method of enhancing the response of *P. putida* or *E. coli* to phenols and substituted phenols by removing the DNA encoding the sensor domain of DmpR or XylR, mutating the respective sensor domain by gene shuffling, ligating the mutant sensor domain into the DNA encoding the respective regulatory protein, and testing the bacteria for enhanced response to said phenols. One would have been motivated to mutate *only* the sensor domain of the regulator proteins of claim 1 because of the teachings of Pavel et al. who taught that the binding specificity of regulatory proteins can be broadened by mutating only the sensor domain and not the DNA binding and transcriptional activation domains. One would have been motivated to use gene shuffling to mutate the sensor domain because of the teachings of Stemmer et al. who taught that by

using gene shuffling, one can create a *combinatorial* library of mutants. One would have a reasonable expectation of success for a method of enhancing the response of *P. putida* or *E. coli* to phenols and substituted phenols by removing the DNA encoding the sensor domain of DmpR or XylR, mutating the respective sensor domain by gene shuffling, ligating the mutant sensor domain into the DNA encoding the regulatory protein, and testing the bacteria for enhanced response to said phenols because of the results of Pavel et al., Willardson et al., Minshull et al., and Cadwell et al. Therefore, claim 8, drawn to a method of enhancing the response of the recited bacteria to phenols and substituted phenols by removing the DNA encoding the sensor domain of the recited regulator protein, mutating the sensor domain by gene shuffling and ligating the mutant sensor domain into the DNA encoding the regulatory protein, and testing the recited bacteria for enhanced response to said phenols would have been obvious to one of ordinary skill in the art.

Applicants' arguments have been addressed in a previous Office action (Paper No. 7). In view of applicants' lack of response to the examiner's response to applicants' arguments, the rejections are maintained for the reasons of record.

Conclusion

10.   No claim is in condition for allowance. All claims are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (703) 308-3934. The examiner can normally be reached Monday-Friday from 8:00 am to 4:30 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX number for this Art Unit is (703) 308-4242. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman, Ph.D.

REBECCA E. PROUTY
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